5926

Influence of Altered Fatty Acid Composition on Resistance of *Listeria monocytogenes* to Antimicrobials

V. K. JUNEJA[1] and P. M. DAVIDSON[2*]

ABSTRACT

The sensitivity of *Listeria monocytogenes* Scott A and ATCC 19114 to antimicrobial compounds was altered when bacterial membrane lipid composition was modified by growth in the presence of added fatty acids. Analysis of cellular fatty acid composition by gas-liquid chromatography indicated that *L. monocytogenes* Scott A cells contained 0.97, 2.32, 0.81, and 0.72% (relative) of C14:0, C16:0, C18:0, and C18:1, respectively. In the presence of exogenously supplied C14:0, C16:0, C18:0, and C18:1, the percentages increased to 14.03, 30.92, 16.30, and 27.90%. Average MICs for *L. monocytogenes* Scott A and ATCC 19114 to sodium chloride, tertiary butylhydroquinone, methyl paraben, and propyl paraben were 10.0%, 81, 1406, and 544 μg/ml, respectively. Growing either strain in the presence of 50 μg/ml of either exogenously added C14:0 or C18:0 fatty acids increased their resistance to the four antimicrobial compounds. However, growth in the presence of C18:1 led to increased sensitivity to the antimicrobial agents. The results indicate that the susceptibility of *L. monocytogenes* to antimicrobial agents is related to the lipid composition of the cell membrane. Consequently, food preservation processes which alter fatty acid composition of *L. monocytogenes* could result in changes in antimicrobial susceptibility.

Listeria monocytogenes is widely distributed in nature and has been isolated from soil, sewage, animal feed, water and vegetation (*17*). The organism is of concern to the food industry due to its implication in disease outbreaks involving food products (*5,7*). Because of the psychrotrophic nature of *L. monocytogenes*, refrigeration alone cannot be relied upon to control the growth of the organism. A potential method of controlling the growth of *L. monocytogenes* during low temperature storage is the use of antimicrobial agents. Davidson and Juneja (*4*) indicated that many antimicrobials approved by the Food and Drug Administration for use in foods have shown little activity against *L. monocytogenes*.

While the mechanisms of resistance of bacteria to antibiotics are well-established, little work has been done on mechanisms of resistance to nonantibiotic antimicrobials. Most research on food antimicrobials has focused on their mechanisms of action against microorganisms. Resistance may be termed intrinsic or acquired (*12*). Intrinsic resistance may be defined as "a natural (innate) chromosomally controlled property of an organism" while acquired resistance results from "genetic changes in a bacterial cell and arise either by mutation or by the acquisition of genetic material from another cell" (*12*). While acquired resistance is undoubtedly important in some circumstances, it is most likely intrinsic resistance which plays the major role in susceptibility of foodborne microorganisms to food antimicrobials. For gram-negative microorganisms, the outer membrane plays an important role in the intrinsic resistance against preservative antimicrobials (*9*). In contrast, Russell (*12*) identified no factors which might lead to intrinsic resistance among gram-positive microorganisms.

Research on resistance of various gram-positive microorganisms has focused primarily on the lipid composition of the cytoplasmic membrane. Since the cell walls of gram-positive bacteria have large exclusion limits, there is probably little or no screening effect by these cellular structures (*12*). Many antimicrobial preservatives used in foods have some amount of hydrophobic character. Therefore, it seems logical that lipid in the cell membrane may have an effect on the ability of these compounds to enter the cell and cause inhibition. There have been a number of studies which have related cytoplasmic membrane lipid composition with inhibition or resistance (*1,2,6,11,13,16*).

The objectives of this study were to change the fatty acid composition of *L. monocytogenes* by growing cells in the presence of selected fatty acids and determine if changes in fatty acid composition influence cellular susceptibility to antimicrobial agents.

MATERIALS AND METHODS

Listeria monocytogenes strains Scott A (serotype 4b) and ATCC 19114 (serotype 4a) were used in the study. The strains were obtained from The University of Tennessee Food Technology and Science Department stock cultures. Cells were grown on tryptose phosphate agar (Difco, Detroit, MI) slants at 35°C for 24 h and stored at 4°C. The cultures were transferred periodically to maintain viability. Tryptose phosphate broth (TPB; Difco) was used to grow

[1]*Present address: Eastern Regional Research Center, U.S. Department of Agriculture, Agricultural Research Service, Philadelphia, Pennsylvania 19118.*

[2*]*Present address: Department of Food Science and Toxicology, Food Research Center, University of Idaho, Moscow, Idaho 83843.*

Mention of brand or firm names does not constitute an endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

cultures prior to all experiments. Peptone (0.1% wt/vol) was used as a diluent for cultures grown in TPB.

Modification of lipid composition

Bacterial cells were grown in TPB containing (50 µg/ml) exogenous fatty acids (e.g., C14:0, C18:0, or C18:1) at 35°C for 24 h and harvested by centrifugation using an Eppendorf centrifuge model 5413 (Brinkmann Instruments, Inc., Westbury, NY). In order to determine the fatty acids present in unmodified cells and in cells grown in the presence of fatty acids, the following procedure was used: the cell pellet was washed with peptone water (0.1%, wt/vol) containing 0.1% Tween 80 and centrifuged. The purpose of the Tween 80 was to remove any added fatty acid that was not incorporated into the bacterial cell membrane. The cell pellets were then washed with peptone water (without Tween 80) and centrifuged; this washing and centrifugation was repeated three times. Thereafter, cells were grown in TPB (in the absence of exogenous fatty acid) for 24 h followed by harvesting and washing with peptone water. The fatty acid composition was determined using a modification of the MIDI Automated Microbial Identification System (*14*). Methyl esters were analyzed using a Hewlett-Packard (HP; Kennett Square, PA), 5890 Series II Gas Chromatograph on a SPB-1 fused silica capillary column (Supelco, Inc., Bellefonte, PA). Major fatty acids were identified by comparison of retention times with those of a pure bacterial fatty acid methyl esters standard (Supelco, Inc.). The chromatographic conditions were as follows: column temperature programmed from 150 to 250°C at 4°C/min; carrier gas flow rate linear velocity, 20 cm/s of helium; injection temperature, 250°C; detector, flame ionization at 280°C; sample size, 5 µl of sample extract, 1 µl of standard.

Resistance of lipid adapted strains

Listeria monocytogenes strains Scott A and ATCC 19114 with altered lipid composition were evaluated for resistance to methyl and propyl parabens, tertiary butylhydroquinone (TBHQ) and sodium chloride by determining the minimum inhibitory concentration (MIC) and inactivation over time. The MIC was determined using a broth dilution assay (*3*). To study inactivation of *L. monocytogenes* over time, cells grown in the presence or absence of exogenous fatty acid were suspended in 0.1% peptone water containing methyl paraben (1500 ppm), propyl paraben (1000 ppm), methyl and propyl paraben (1:1 at 0.1%), sodium chloride (10%), or TBHQ (200 ppm) and incubated at 35°C. Samples were taken at 0, 15, 30, 45, 60, and 180 min, serially diluted, and plated on tryptose phosphate agar. The colony counts were determined after incubation for 48 h at 35°C.

Statistical analysis

Analysis of variance was done by using SAS (SAS Institute Inc., Cary, NC). Tukey's Multiple-Range Test was used to identify significant differences ($p < 0.05$) among means (*15*).

RESULTS AND DISCUSSION

Modification of lipid composition

Modification of the lipid composition was achieved when *L. monocytogenes* Scott A was grown in the presence of exogenous fatty acids (Table 1). In comparison to the untreated control, the relative percentages of C14:0, C16:0, C18:0, or C18:1 incorporated into *L. monocytogenes* cells was considerably higher when those fatty acids were supplied as part of the growth medium.

Resistance of lipid adapted strains

For *L. monocytogenes* Scott A with increased C14:0 or C18:0 fatty acids, the MIC for methyl paraben was significantly increased (1637.5 µg/ml and 1900 µg/ml, respectively) compared to the control cells (1412.5 µg/ml) (Table 2). In contrast, the MIC for methyl paraben of cells grown in the presence of C18:1 was significantly decreased (1162.5 µg/ml) compared to the control. The same pattern was observed for propyl paraben, TBHQ, and sodium chloride (Table 2). The only exception was cells grown in the presence of C18:1 where the decrease in MIC for TBHQ was not significant ($p > 0.05$). Similar results were obtained with *L. monocytogenes* ATCC 19114 (Table 3).

TABLE 1. *Mean relative percentages[a] of fatty acids in* L. monocytogenes *strain Scott A[b].*

Fatty acid	Control	Exogenous fatty acid C14:0	C16:0	C18:0	C18:1
14:0	0.97	14.03	-	-	-
Br-15:0	41.86	48.39	22.98	41.90	34.98
16:0	2.32	-	30.92	-	1.31
Br-17:0	27.72	32.05	7.47	24.69	31.83
18:0	0.81	-	3.42	16.30	-
18:1	0.72	-	-	-	27.90

[a] Means represent two replications.

[b] Grown in tryptose phosphate broth containing exogenous fatty acids (50µg/ml) at 35°C and subsequently washed with peptone water containing 0.1% Tween 80; only major fatty acids are shown.

Results of the MIC studies for control cells of *L. monocytogenes* were comparable to those reported by Payne et al. (10). In that study, methyl paraben, propyl paraben, and TBHQ were found to have MICs of >512, 512, and 64 µg/ml, respectively. Slight differences in MIC values between the present study and those reported previously were probably due to growth conditions or concentration intervals.

No inactivation of control *L. monocytogenes* Scott A was achieved over a 3-h period in the presence of 1500 µg/ml methyl paraben, 1000 µg/ml propyl paraben, a mixture of 0.1% methyl and propyl paraben, or 10% sodium chloride. The number of viable cells of the control remained constant during the 3-h incubation period at 35°C (data not shown). This was most likely due to the slow-acting nature of these compounds.

In contrast, the control was rapidly inactivated in the presence of TBHQ (200 µg/ml) (Figs. 1 and 2). The data for the inactivation of *L. monocytogenes* over time have been expressed as log of the ratio of count at time t (N) and initial count (N_0). For control cells of *L. monocytogenes* Scott A, the ratio decreased by 1.94 ($\log_{10}$ 7.88 CFU/ml to $\log_{10}$ 5.94 CFU/ml) within 45 min and by 4.23 ($\log_{10}$ 3.65 CFU/ml) at 3 h (Fig. 1). In comparison to the control, *L. monocytogenes* Scott A cells grown in the presence of C18:1 were more susceptible, the ratio decreasing by 4.25 ($\log_{10}$ 7.23 CFU/ml to $\log_{10}$ 2.98 CFU/ml) in 3 h (Fig. 1). In fact, a sharp decline (99%) in the number of survivors was observed in 15 min. Conversely, cells grown in the presence of C14:0 and C18:0 fatty acids were more resistant to 200 µg/ml TBHQ than the control. For *L. monocytogenes* Scott A cells grown in the presence of C14:0, the ratio decreased by 2.67 ($\log_{10}$ 6.97 CFU/ml to $\log_{10}$ 4.30 CFU/ml) in 3 h. Cells grown with C18:0 displayed the greatest resistance. A decrease of only